COMPARATIVE SUSCEPTIBILITIES OF *ANOPHELES QUADRIMACULATUS* MUTANTS TO *PLASMODIUM YOELII*

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ABSTRACT. Four homozygous mutant stocks and a wild-type stock (ORLANDO) of *Anopheles quadrimaculatus* were compared for susceptibility to *Plasmodium yoelii*. The mutant stocks, rose eye (*ro*) and red stripe (st^{rd}), had the same level of susceptibility as the control stock (ORLANDO), but Q2 (homozygous for brown body and nonstripe) and rose eye Q2 (roQ2), were significantly less susceptible. Body size of the adult mosquitoes had no effect on susceptibility.

INTRODUCTION

While studying the susceptibility of a laboratory colony (ORLANDO strain) of *Anopheles quadrimaculatus* (Say) to infection with a rodent malaria, it was noted that the infectibility of the mosquitoes varied considerably. Some females had heavy infections, but other females from the same cohort did not become infected or developed only light infections. Variability in the susceptibility of anopheline mosquitoes to infection by *Plasmodium* species has been an accepted norm by researchers engaged in such studies. The level of susceptibility of a particular strain or species is affected by both hereditary and environmental factors (Curtis and Graves 1983).

Three mutants of *An. quadrimaculatus* have been described by Mitchell and Seawright (1984a, 1984b). Because the stocks homozygous for these genetic markers were established by inbreeding, it is likely that other genes in these stocks were simultaneously fixed in homozygous condition. We therefore suspected that the selection for susceptible or refractory genes could have been done inadvertently, as documented previously for *Anopheles albimanus* Wiedemann by Warren et al. (1975, 1977). In the present paper, we compare the susceptibility to infection with *Plasmodium yoelii* of four mutant strains and a wild type, laboratory strain. The effect of larval rearing density on susceptibility was also investigated.

MATERIALS AND METHODS

The strains of *Anopheles quadrimaculatus* tested were:

(1) ORLANDO—This wild type strain has been maintained in the laboratory for over forty years.

(2) red stripe (st^{rd})—This strain is homozygous for the dominant trait, red stripe, on the right arm of chromosome 3. It was established by crossing ORLANDO females to red stripe male progeny of gravid mutant females collected at Lake Panasoffkee, Florida. The red stripe character is expressed as a bright red dorsal stripe in the larval, pupal and adult stages; st^{rd} is codominant with white stripe (*st t*) and dominant to nonstripe (*st*) in an allelic series.

(3) Q2—This strain is homozygous for the recessive traits brown body (*bw*) on the left arm of chromosome 2 and nonstripe. The phenotype of *bw* homozygotes is a melanotic body color evident in the larval, pupal and adult stages. Nonstripe (the absence of a dorsal stripe) is visible only in larvae and pupae. This mutant strain was isolated from the ORLANDO colony and is also homozygous at 14 enzyme loci.

(4) rose eye (*ro*)—This mutant was induced by treating ORLANDO mosquitoes with ethyl methane sulfonate (EMS). The rose eye color is an X-linked recessive trait expressed as pink eye color in larvae, pupae, and adults.

(5) roQ2—This strain is homozygous for the recessive traits, rose eye, brown body, and nonstripe.

The mosquitoes used for controls were from the ORLANDO colony of *An. quadrimaculatus*. Rearing procedures for this strain were similar to those reported by Bailey et al (1980). Approximately 5,000 eggs were hatched in 3 oz cups containing 75 ml of infusion water (0.04% liver powder and brewer's yeast (1:1)). After 24 hours, newly-hatched larvae were placed in plastic trays (56 × 43 × 7.5 cm.) with 3 liters of water and 50 ml of a 2% slurry containing liver powder and brewer's yeast (1:1). Larvae were again fed 50 ml of 1:1 on the third day after hatching and trays were dusted with finely-ground Purina® Hog-Fish Chow on the fourth day. Thereafter, 100 ml of a 2% infusion containing liver powder, brewer's yeast and hog

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supplement (1:1:1) was fed daily until pupation.

Mutant strains were reared in a manner similar to the ORLANDO strain except that larvae were fed a 2% slurry of Tetramin® Baby E fish food and brewer's yeast (2:1), as required, and mosquitoes were reared at a low density (approximately 200 individuals per liter as compared to 1,300 per liter for ORLANDO controls).

To test for possible variations in susceptibility to infection due to differences in larval rearing densities, one group of ORLANDO mosquitoes was reared in the same manner as the mutant strains. This group was designated as CMRLM, an acronym for Control Mosquitoes Reared Like Mutants. Cohorts of 65 newly-emerged adults (less that 24 hours old) from the ORLANDO strain reared at high density and the mutant Q2 strain were weighed for comparison.

Syrian hamsters with induced infections of rodent malaria (*P. yoelii*) were anesthetized with sodium pentobarbitol and mosquitoes were allowed to feed on parasitemia day 3, 4 or 5. Both mutants and controls were fed on the same malarious hamster within a few minutes of each other.

Adult mosquitoes were maintained and fed in Plexiglas® cages, 21 cm on a side, with one end screened and the other end covered with a cheesecloth sleeve. Feeders made from vials and cotton wicks were kept in the cages. The feeders contained a 10% sucrose solution prior to blood feeding and thereafter contained a 10% sugar solution with 0.1% methionine. Adult mosquitoes were given infective blood meals when about 4 days of age. Following the feeding, all mosquitoes except engorged females were removed from the cage.

The engorged females were incubated in an environmental chamber at 20°C and 95% relative humidity. One week after the initial blood feeding a supplementary feeding was given using an uninfected hamster.

On about the 13th day after the initial blood feeding, mosquitoes were dissected to determine infection prevalences. The oocysts on each gut were counted and scored using the following categories: 0 oocysts per gut, 1–25, or more than 25. Equal numbers of mutants and controls were dissected. When more than one strain of mutant was fed on a given day, only one cage of control mosquitoes was fed. Sample size was variable; therefore, the number of control mosquitoes examined was equal to the largest sample size of the four mutant types. For the other mutant types, a direct comparison was made in consecutive order with the control strain, e.g., if 50 mutant mosquitoes were dissected then the comparison was made with the first 50 control mosquitoes. Results were summarized in two-way contingency tables, and Chi Square tests were done (SAS, Proc Means).

RESULTS AND DISCUSSION

ORLANDO control mosquitoes reared at high density were smaller than the mutant strains and the CMRLM group reared at low density. The mean weight of control adults (1.7 mg) was approximately one-half that of Q2 adults (3.2 mg). However, infection prevalences and oocyst intensities of ORLANDO mosquitoes reared under different conditions (CMRLM vs controls) were identical (Tables 1 and 2). In addition, no mutant strain had higher prevalences of infection or larger numbers of oocysts per infected gut than the corresponding control group. It is therefore concluded that adult size had no effect on susceptibility to infection or oocyst intensity. One may have expected larger females to become more heavily infected due to the fact that they are able to ingest more parasitized blood (Jeffery 1956). Other researchers, however, have also found that the size of the female had no influence on susceptibility to malaria infection and that only a weak relationship existed between the quantity of blood ingested and the number of oocysts formed (Hovanitz 1947, Ward 1963).

In two of the mutant strains tested, i.e. red stripe and rose eye, both the susceptibility and incidence of heavy infection were not significantly different from the ORLANDO controls (Table 1 and 2). The Q2 and roQ2 strains, both of which are homozygous for brown body, were significantly less susceptible. Since the rose eye (*ro*) type was susceptible to infection with *P. yoelii* and the roQ2 strain was highly refractory, it is doubtful that the X chromosome plays a significant role in the refractory mechanism. It follows then that the gene(s) responsible for the refractory state are probably autosomal. It is unclear whether that the refractory state is related to the melantic phenotype, even though the strains homozygous for brown body tended toward refractoriness. Warren et al. (1979) found that pupal coloration and other color variants were not directly associated with susceptibility of *An. albimanus* to infection with human malaria. It is possible that the Q2 strain is refractory as a matter of chance and not as a matter of a pleiotropic effect of the *bw* locus.

Although it has been recognized for a long time that vector competence has a genetic basis, information concerning the genetic mechanisms controlling susceptibility to malaria infection in anopheline mosquitoes is limited.

Table 1. Comparison of control *Anopheles quadrimaculatus* with four mutant strains for susceptibility to infection with *Plasmodium yoelii*.

	CMRLM[1]	Mutant strains			
		rose eye	red stripe	Q2	rose eye Q2
Paired lots	8	18	15	14	12
Total controls dissected	83	202	238	124	156
Total mutants dissected	83	202	238	124	156
Controls negative for oocysts	11	13	17	31	16
Mutants negative for oocysts	11	14	19	69	42
Chi square probability	1.0	0.84	0.72	0.0001**	0.0002**

[1] CMRLM are control mosquitoes reared under less crowded conditions (see text).
** The difference in proportion of mutants vs. controls infected is statistically highly significant.

Table 2. Comparison of control *Anopheles quadrimaculatus* with four mutant strains for susceptibility to heavy vs. light infections with *Plasmodium yoelii*.

	CMRLM[1]	Mutant strains			
		rose eye	red stripe	Q2	rose eye Q2
Total controls infected	72	189	221	93	140
Total mutants infected	72	188	219	55	114
Controls heavily infected (more than 25 oocysts)	69	165	186	67	106
Mutants heavily infected	70	155	191	18	49
Chi square probability	0.65	0.19	0.36	0.0001**	0.0001**

[1] CMRLM are control mosquitoes reared under less crowded conditions (see text).
** The difference in proportion of mutants vs. controls that became heavily infected (as opposed to lightly infected) is statistically highly significant.

There are many variables associated with the host-parasite-mosquito system, making it very difficult to measure accurately the phenotypes and assign them unambiguously to either refractory or susceptible classes (Curtis and Graves 1983). *Anopheles quadrimaculatus*, however, is a good candidate for further genetic analysis of the refractory mechanism since interstrain variations in susceptibility do exist and genetic markers are available on both autosomes as well as the X chromosome in this species.

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